ANTITOXIN FORMATION BY CELLS TRANSPLANTED INTO AN IRRADIATED ANIMAL

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 55, No. 1, pp. 61-65, January 1963
Original article submitted January 16, 1962

The method of study of certain problems in immunology by transferring cells from an immunized donor to an immunologically inactive recipient (new-born, irradiated, or tolerant animals) has given great opportunities to the research worker.

The first observations were made in 1899 [3]; many reports of investigations in vitro [1, 4, 14, 15, 21, 22, 24, 26] and in vivo [2, 5-12, 18, 19, 23, 25] were subsequently published. It was demonstrated that the spleen, lymph glands, and bone marrow could form antibodies in the organism of the recipient, and various principles governing this process were established. However, all the researches cited above were carried out on corpuscular bacterial antigens, and only the work of Stavitsky [16, 17, 20, 26] and his associates dealt with antitoxic immunity.

The aim of the present research was to study, by means of the usual subcutaneous method of immunization, the following problems: 1) which cells, when transplanted into another organism, can form antitoxin; 2) the significance of the phase of antibody-formation; 3) the immunological response of the cells to stimulation by two unrelated antigens; 4) whether transplanted cells are capable of giving a secondary immunological response.

EXPERIMENTAL METHOD

The donor animals were immunized subcutaneously: guinea pigs twice at an interval of 30 days with adsorbed diphtheria toxoid (30 lf + 60 lf) or once with crude toxoid (60 lf); rabbits simultaneously with two antigens – adsorbed diphtheria toxoid and typhoid formol vaccine (strain Ty 4446), either once (60 lf toxoid + $500 \cdot 10^6$ bacterial cells of vaccine) or repeatedly over a period of 30 days: twice with toxoid (30 lf + 60 lf) and three or four times with vaccine ($500 \cdot 10^6$ to $2 \cdot 10^9$ bacterial cells). Donors were sacrificed at certain intervals after the last injection of antigen and tissue was taken from them.

Cell suspensions were prepared from the spleen, lymph glands (submandibular, mesenteric, inguinal, and popliteal), and bone marrow. The organs were pressed into a glass homogenizer, the marrow was washed out of the long bones, and the cells were suspended in Earle's solution. After washing by centrifugation (1000-2000 rpm) to remove fat and possible antigen, the cells were resuspended and injected intravenously into the recipient. Control cells were killed by heat (56°, 30 min). The total number of cells injected was as follows: into the rabbit – $250 \cdot 10^6$ marrow cells, of which 90% were living, $300 \cdot 10^6$ spleen cells, of which 60% were living, and $350\text{-}1000 \cdot 10^6$ lymph gland cells, of which 60% were living; into the guinea pig – $200 \cdot 10^6$ marrow cells, and $100\text{-}150 \cdot 10^6$ each of the spleen and lymph gland cells.

The recipients were irradiated with roentgen rays in the following doses: guinea pigs – 200 and 525 r, rabbits – 550 and 800 r. From 2 to 4 hr later they were injected intravenously with suspensions of the corresponding cells. In addition to spleen and lymph gland cells from "immune" animals, guinea pigs irradiated with a lethal dose also received injections of $200 \cdot 10^6$ normal bone marrow cells. In the interval between the 3rd and 32nd days, depending on the experiment, blood samples were taken and tested for antibodies.

EXPERIMENTAL RESULTS

Three experiments were devoted to the study of the inductive phase of antibody-formation: cells were taken from donors 24 hr after a single immunization. In two experiments on guinea pigs irradiated with 200 or 525 r (ab-

Antitoxin Formation in Irradiated Guinea Pigs by Transplanted Cells

Dose of irradiation of recipient (in r)	Tissue	No. of animals	Day after transplantation			
			4th	7th	10th	15th
			Antitoxin titer (in antitoxin units)			
200	Bone marrow	5	—	—	—	—
	Spleen	5	—	0,023	—	—
	Lymphocytes	4	—	0,02	0,038	—
525	Bone marrow	9	—	0,008	—	—
	Spleen	7	0,013	0,028	0,076	—
	Lymphocytes	7	—	—	0,016	—
Control	Killed cells	9	—	—	—	—

solutely lethal dose), and in one experiment on rabbits irradiated with 550 r, for a period of 30 days after transplantation of homologous cells from the spleen, lymph glands, or marrow we were unable to detect the presence of diphtheria antitoxin, or agglutinins (in the rabbits), in the recipients. Transplantation of killed cells naturally also failed to produce antibodies.

The ability of cells taken during the reproductive phase to produce antibodies was studied in four experiments (see table and Figs. 1 and 2).

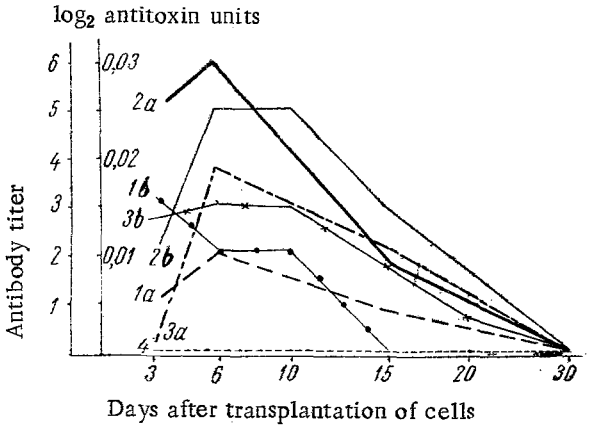

Fig. 1. Antibody formation in irradiated (550 r) rabbits by transplanted cells: marrow (1); spleen (2); lymph gland (3); killed by heat (4); a) antitoxin, b) agglutinins. Mean titers are given. Each group consisted of 4 rabbits.

Experiments on guinea pigs showed that all the transplanted tissues could form antitoxin, and this power was more evident in the recipients irradiated with a lethal dose. In this case the marrow, too, produced detectable quantities of antibodies. In no animal was antitoxin found after transplantation of killed cells. The formation of antibodies, against both antigens used, by the transplanted cells was observed in the rabbits (Fig. 1). Tissues were obtained from the donors 7 days after the last injection of antigen, i.e., at the time when their antibody titer reached its maximum. The recipient's blood contained antibodies on the third day. Their titer reached its maximum on the 6th day, fell sharply on the 15th day, and none could be found on the 30th day. The pattern of formation of antibodies to both antigens was identical.

In another experiment on rabbits (Fig. 2) substantially the same results were obtained. The maximum of antitoxin formation occurred on the 6th-10th day, with a sharp fall on the 15th day and absence of antitoxin on the 18th day (Fig. 2, a). The pattern of agglutinin formation was identical (Fig. 2, b). On the 15th day after transplantation of the cells, the recipients received injections of diphtheria toxoid (60 lf) and of formol vaccine ($1 \cdot 10^9$ bacterial cells). In response to the injection of antigens (see Fig. 2), no antitoxin was formed. Conversely, the aggultinin titer rose equally in all the animals, even in those receiving injections of killed cells.

The experimental findings shed some light on the problems which we had undertaken to investigate:

1. After the usual subcutaneous immunization (when there is no intention to produce antibody formation in a regional gland), all tissues used for transplantation can form antibodies (both agglutinins and antitoxins) with a maximum of the titer on the 6th day, followed by a rapid fall until the 15th-20th day. Although, according to some writers [2], bone marrow cells are incapable of producing antibodies, the results of many investigations [5, 16, 20] are in agreement with those we have described.

In his researches, Harris [6-9] gave a single injection of antigen, and the time of antibody formation and of the maximal titer on the 6th day was associated with contact between antigen and cell, and not with the time of transplantation. In our experiments the antibody titer in similar animals also reached its maximum on the 6th day, but

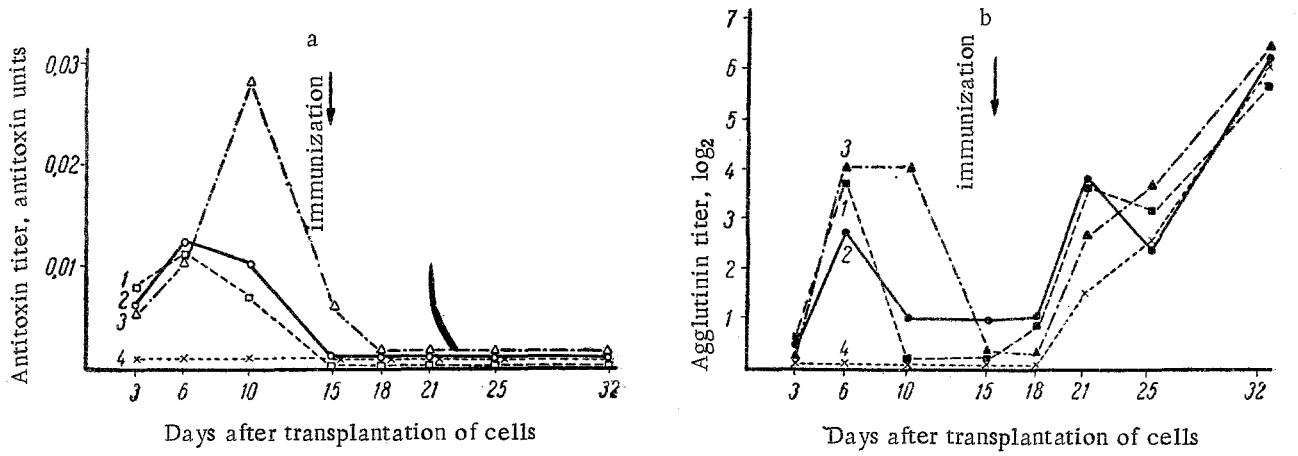

Fig. 2. Antibody formation in irradiated (800 r) rabbits by transplanted cells: marrow (1); spleen (2); lymph gland (3); killed by heat (4); a) antitoxin, b) agglutinins. Mean titers are given. Each group consisted of 6 rabbits. The arrows denote immunization of recipients with 60 lf of diphtheria toxoid and $1 \cdot 10^9$ bacterial cells of typhoid vaccine.

this was after transplantation, i.e., on the 13th day after the last injection of antigen. Consequently, it may be suggested that the transplanted cells must become "adapted" to the host organism, and proliferate; antibody production [23] takes place cells of one or two generations. The following reasons may be suggested for the cessation of antibody production by the transplanted cells:

a) the cells begin to compete with the host, which regains its immunological resistance, and are eliminated from the body. Elimination of homologous cells takes place on the 20th day [11];

b) the cells in general cease their activity at a definite time, although it is uncertain why this may occur at the same time in the transplanted tissue both after a single immunization of the donor, the cells being taken 7-10 hr after the injection, and after hyperimmunization of the donor, the cells being taken 7 days after reimmunization;

c) the cessation of antibody production is the result of immunological paralysis of the transplanted cells after meeting a high concentration of foreign antigen of the host. It has been shown, for example [20], that cells will function well in an isologous organism for 25 days, in a homologous organism they cease to produce antibodies significantly sooner, while a heterologous transplant generally speaking will not form antibodies [11, 19].

2. The cells of all the investigated organs, taken in the inductive phase, are incapable of producing either antitoxin or agglutinins. This is confirmed by the work of Trnka and Sterzl [23], who also found that antibody formation is absent if these cells were not placed in contact once again with the antigen in vitro. Some workers assert the contrary [9], but in their experiments they used the regional lymph glands. On the other hand, in the reproductive phase, all the tissues investigated in conditions of hyperimmunization form antibodies, although not necessarily in all animals.

3. It was found (see Figs. 1 and 2) that an organ is capable of responding by the formation of antibodies to unrelated organs. Antibody formation follows a parallel course and reaches its maximum at the same time. Consequently, the administration of both corpuscular and soluble antigen involves the same organs in antibody formation. We cannot conclude from these results that one cell is capable of forming two different antibodies, although this suggestion may be made.

4. In the work we are discussing, immunization of the recipient on the 15th day after transplantation of the cells did not give a secondary immunological response. During the period of observation (17 days) antitoxin was not formed in the recipients' blood in detectable amounts (see Fig. 2, a), but agglutinins (see Fig. 2, b) were found in equal and parallel titers in recipients of both living and killed cells. The character of the rise in the antibody titers was similar to that in the primary response. Confirmation of this point may be found in the literature [17], although the reason for the failure of the experiments was presumably that at the time of immunization very few donors' cells, or perhaps none at all, were left in a viable state. A secondary, anamnestic response (work with isologous animals) was obtained as long as 14 days after transplantation of the cells [2, 5].

SUMMARY

An inquiry was made into some aspects of antitoxic immunity by using the cellular transplantation method. Guinea pigs and rabbits were used for the experiment. The recipients were irradiated: guinea pigs with 200-525 r,

rabbits with 550 - 800 r. The donors were immunized with the diphtheria toxoid and typhoid formol-vaccine. Cells of the spleen, lymph nodes and bone marrow were transplanted. The following was revealed as a result of the work carried out: the cells of all the tissues used were capable of producing the antibodies in the organism of the irradiated recipient; one and the same organ responds by antibody production to both antigens used: production of both antibodies pursued a parallel course with the maximum on the 6-10th day; on the 15th-20th day they were undetectable. Recipients gave no anamnestic reaction to the antigen injection 15 days after the cell transplantation.

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